# 21 ETHYLENE GLYCOL AND PROPYLENE GLYCOL QUANTIATION AND CONFIRMATION BY GCMS

## 21.1 Summary

21.1.1 In an aliquot of biological samples, ethylene glycol and propylene glycol are derivatized with phenylboronic acid to form their respective boronate esters. The samples are then made basic with potassium hydroxide and extracted with methylene chloride. Following an acid wash, the extracts are concentrated and injected onto a GCMS for simultaneous quantitation and confirmation of both ethylene and propylene glycol.

## 21.2 Specimen Requirements

21.2.1 Approximately 2 mL of biological fluid or tissue dilution/homogenate

## 21.3 Reagents and standards

21.3.1 Ethylene glycol

21.3.2 Propylene glycol

21.3.3 1,2-butanediol

21.3.4 Acetone

21.3.5 Hexane

21.3.6 Methylene chloride

21.3.7 Phenylboronic acid

21.3.8 Potassium hydroxide

21.3.9 Concentrated hydrochloric acid

21.3.10 Sodium sulfate, anhydrous

## 21.4 Solutions, internal standard, calibrators and controls

21.4.1 1% phenylboronic acid solution: Weight 1 g phenylboronic acid. Transfer to 100 mL volumetric flask and QS to volume with acetone. Store in refrigerator, stable for up to 1 year.

21.4.2 0.1% phenylboronic acid solution: Pipet 10 mL of 1% phenylboronic solution into a 100 mL volumetric flask and QS to volume with acetone. Prepare fresh daily.

21.4.3 0.1N potassium hydroxide. Weigh 2.8 g potassium hydroxide and transfer to 500 mL volumetric flask. QS to volume with $dH_20$.

21.4.4 Ethylene glycol stock solution (200 mg/mL): Weigh 1 g ethylene glycol. Transfer to 5 mL volumetric and QS to volume with $dH_20$. Store in refrigerator, stable for up to 1 year.

21.4.5 Propylene glycol stock solution (200 mg/mL): Weigh 1 g propylene glycol. Transfer to 5 mL volumetric flask and QS to volume with $dH_20$. Store in refrigerator, stable for up to 1 year.

21.4.6 1,2-butanediol internal standard stock solution (5 mg/mL): Weigh 250 mg 1,2-butanediol. Transfer to 50 mL volumetric flask and QS to volume with acetone. Store in refrigerator, stable for up to 1 year.

21.4.7 Ethylene glycol/propylene glycol working solution (80 mg/mL): Pipet 2 mL each of 200 mg/mL ethylene glycol and propylene glycol stock solutions into a 5 mL volumetric flask. QS to volume with $dH_20$. Prepare fresh daily.

21.4.8 To prepare the calibration curve, pipet the following volumes of the 80 mg/mL ethylene glycol/propylene glycol working solution into appropriately labeled 16 x 125 mm screw cap test tubes. Add 2 mL blank blood to obtain the final concentration listed below.

| Amount of working standard (µL) | Final concentration of ethylene glycol/propylene glycol (mg/L) |
|---|---|
| 100 | 4000 |
| 50 | 2000 |
| 20 | 800 |
| 10 | 400 |
| 5 | 200 |

21.4.9 Controls

21.5.9.1 Negative control. Blood bank blood (or comparable) determined not to contain ethylene glycol or propylene glycol.

21.5.9.2 Positive control. Control may be from external source or prepared in house using ethylene glycol and propylene glycol from different manufacturer or lot numbers.

**21.5 Apparatus**

21.5.1 Gas chromatograph with mass selective detector

21.5.2 Test tubes, 16 x 125 mm screw cap tubes, borosilicate glass with Teflon caps

21.5.3 Test tubes, 13 x 125 mm screw cap tubes, borosilicate glass, conical botttom

21.5.4 GC autosampler vials with inserts

21.5.5 Evaporator/concentrator

21.5.6 Vortex mixer

21.5.7 Centrifuge capable at 2,000-3,000 rpm

21.5.8 GC/MSD parameters. Conditions may be changed to permit improved performance

21.5.8.1 Acquisition mode: scan (50-550 amu)

21.5.8.1.1 Major ions for ethylene glycol phenylboronate ester are 91, 118 and 148

21.5.8.1.2 Major ions for 1,2-butanediol phenylboronate ester are 91, 147 and 176

21.5.8.1.3 Major ions for propylene glycol phenylboronate ester are 91 and 147

21.5.8.2 Column: HP5 MS 25 m x 0.25 mm x 0.25 µm

21.5.8.2 Detector Temperature: 280° C

21.5.8.1 Oven Program

- Equilibration time: 0.50 minutes
- Initial temp 1: 80° C
- Initial time 1: 2 minutes
- Ramp 1: 10° C/min
- Initial hold time 1: 1 minute
- Oven final temp 1: 130° C
- Oven ramp 2: 45° C/minute
- Oven hold time 2: 2 minutes
- Oven final temp 2: 280° C
- Run Time: 15 minutes

21.5.8.1 Inlet

- Mode: Split
- Split ratio: 117:1
- Split vent: 100 mL/min
- Temperature: 250° C
- Injection volume: 2.0 µL
- Purge Time: ON at 1.0 minute

**21.6 Procedure**

21.6.1 Prepare 0.1% phenylboronic acid solution.

21.6.2 Label clean 16 x 125 mm tubes appropriately, calibrators, positive and negative controls and case sample ID's

21.6.3 Prepare calibrators and controls.

21.6.4 Add 2 mL case samples into appropriately labeled tubes.

21.6.5 Add 50 µL 1,2-butanediol internal standard stock solution (5 mg/mL) to each tube. Vortex briefly.

21.6.6 Add 0.5 mL $dH_2O$ to each tube. Vortex briefly.

21.6.7 Add 10 mL of 0.1% phenylboronic acid to each tube. Vortex each sample for 30 seconds.

21.6.8 Centrifuge at approximately 2500 rpm for 15 minutes.

21.6.9 Transfer supernatants to clean 16 x 125 mm tubes. Discard bottom layer (blood pellet).

21.6.10 Evaporate supernatants to approximately 3 mL under nitrogen at 50-60° C. The purpose is to remove acetone (from 0.1% phenylboronic acid solution) and leave the aqueous portion, so overheating is not recommended.

21.6.11 Remove samples from evaporator and add 3 mL of 0.1N KOH to each tube.

21.6.12 Add 5 mL methylene chloride to each tube. Vortex for 30 seconds.

21.6.13 Centrifuge at approximately 2500 rpm for 15 minutes.

21.6.14 Transfer upper (aqueous) layer to labeled 16 x 125 mm tubes. Discard bottom (organic) layer.

21.6.15 Acidify samples with concentrated HCl (1-2 drops of acid per tube for sample to reach approximately pH 4).

21.6.16 Add 5 mL methylene chloride to each tube. Vortex for 30 seconds.

21.6.17 Centrifuge at approximately 2500 rpm for 15 minutes.

21.6.18 Discard upper aqueous layers.

21.6.19 Dry bottom organic layer by adding approximately 100 mg (2 small spatulas) of anhydrous sodium sulfate to each sample. Vortex briefly.

21.6.20 Centrifuge at approximately 2500 rpm for 15 minutes.

21.6.21 Transfer methylene chloride layer to labeled conical bottom test tubes.

21.6.22 Evaporate samples to approximately 200 µL under nitrogen at 50-60° C.

21.6.23 Transfer samples to GC autosampler vials and inject 1 µL onto GCMS for analysis.

**21.7 Calculation**

21.7.1 Drug concentrations are calculated by linear regression analysis using ChemStation software based on peak height (or area) ratios versus calibrator concentration.

**21.8 Quality Control and Reporting**

21.8.1 See Toxicology Quality Guidelines

**21.9 References**

21.9.1 H McCurdy and E Solomons. An improved procedure for the determination of ethylene glycol in blood. J Anal Tox 6: 253-254, 1982.